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Conjugated linoleic acid inhibits glucose metabolism, leptin and adiponectin secretion in primary cultured rat adipocytes

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Abstract

Conjugated linoleic acid (CLA) supplementation has been reported to induce insulin resistance in animals and humans, however, the underlying mechanisms remain unclear. The aim of this study was to examine the direct effects of CLA on leptin and adiponectin secretion, two hormones with actions known to influence insulin sensitivity. Isolated rat adipocytes were incubated with CLA (1–200 μM) in the absence and presence of insulin (1.6 nM). CLA inhibited both basal and insulin-stimulated leptin gene expression and secretion (−30 to −40%, $P < 0.05$–0.01). CLA also inhibited basal adiponectin production (−20 to −40%, $P < 0.05$–0.01), but not in the presence of insulin. CLA (50–200 μM) decreased basal glucose uptake ($P < 0.05$–0.01) and significantly increased the proportion of glucose metabolized to lactate ($P < 0.01$). Insulin treatment partially prevented the inhibitory effects of CLA on glucose uptake and induced a significant increase ($P < 0.05$–0.01) in the percentage of glucose metabolized to lactate. A strong inverse relationship was observed between the increase in the anaerobic utilization of glucose and the decreases of both leptin and adiponectin secretion. In addition, lipolysis and the expression of the adipogenic transcription factor PPARγ were decreased by CLA. These results indicate that CLA inhibits leptin and adiponectin secretion and suggest that increased anaerobic metabolism of glucose may be involved in these effects. The inhibition of PPARγ could also mediate the inhibition of adiponectin induced by CLA. Furthermore, the inhibition of leptin and adiponectin production induced by CLA may contribute to insulin resistance observed in CLA-treated animals and humans.

Keywords: Conjugated linoleic acid; Leptin; Adiponectin; Insulin resistance; Cultured rat adipocytes; Peroxisome proliferator-activated receptor γ

1. Introduction

Conjugated linoleic acid (CLA) is a generic term for the positional and geometric isomers of linoleic acid [18:2($n-6$)]. This fatty acid is produced in the rumen of ruminant animals by the fermentative bacteria, *Butyrivibrio fibrisolvens*, which isomerizes linoleic acid into CLA (Kepler et al., 1966). Another source of CLA in ruminants is synthesis *via* Δ 9-desaturase of *trans*-11 octadecanoic acid (Griinari et al., 2000). The major dietary sources of CLA are dairy products as well as ruminant meats such as beef and lamb (Chin et al., 1992). CLA isomers can also be chemically and commercially produced by heating linoleic acid in the presence of alkali or by partial hydrogenation of linoleic acid (Banni, 2002).

CLA has been studied extensively due to its potential beneficial effects in several disease states including cancer (Belury, 2002), atherosclerosis (McLeod et al., 2004), diabetes (Houseknecht et al., 1998), and obesity (Wang and Jones, 2004a). CLA has also been reported to increase immune function (O'Shea et al., 2004). The majority of the studies conducted in animals have demonstrated that CLA reduces body fat mass (Wang and Jones, 2004a,b), although in some studies no effects of CLA on body composition were observed (Mirand et al., 2004). In contrast, the consensus from seventeen published studies in human subjects is that CLA supplementation does not affect body weight or body composition (Tricon et al., 2005).

CLA supplementation has also been reported to be associated with deleterious effects including the development of insulin resistance and hyperinsulinaemia both in mice and humans (Tsuboyama-Kasaoka et al., 2000; Riserus et al., 2002, 2004).

Several studies have shown that CLA supplementation decreases circulating leptin concentrations (Medina et al., 2000;

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Yamasaki et al., 2003). It has been suggested that CLA-induced reductions in circulating leptin levels are associated with the reductions in fat mass caused by CLA (Wang and Jones, 2004a,b), however, in the study by Medina et al. (2000), fat mass was not reduced by CLA. Therefore, CLA might also directly affect leptin production. For example, incubation of 3T3-L1 adipocytes with *trans*-10, *cis*-12-CLA (t10c12 isomer: the CLA isomer suggested to be most effective at reducing fat mass in animals) inhibited leptin secretion and expression (Kang and Pariza, 2001). However, another more recent study found that the t10c12 isomer increased leptin gene expression in cultures of stromal vascular cells (SV cells) containing newly differentiated human adipocytes (Brown et al., 2004).

Adiponectin is a hormone specifically secreted by adipose tissue with important metabolic effects (Havel, 2004). Plasma adiponectin concentrations are decreased in obesity and are positively associated with whole-body insulin sensitivity (Stefan et al., 2002a). Low adiponectin levels have been shown to precede and predict the onset of insulin resistance, type 2 diabetes and cardiovascular diseases (Hotta et al., 2001; Stefan et al., 2002b; Kumada et al., 2003). Two recent *in vivo* studies in mice reported that the hyperinsulinaemia induced by CLA treatment was preceded by decreases in leptin and adiponectin plasma levels (Ohashi et al., 2004; Poirier et al., 2005). These data suggest a potential direct inhibitory action of CLA on adiponectin production, however, the direct effect of CLA on adiponectin secretion by adipocytes has not been investigated.

The aim of the present study was to investigate the *in vitro* effects of CLA on adiponectin and leptin production from primary rat adipocytes. Isolated adipocytes were attached to a collagen matrix and cultured with CLA for 96 h. This system simulates normal basement membrane attachment and maintains adipocyte differentiation (Mueller et al., 1998), resulting in a more physiological environment compared to "free-floating" adipocytes.

The effects of CLA on basal and insulin-stimulated glucose utilization, lactate production and lipolysis were also studied. A number of studies have demonstrated that leptin production is modulated by insulin responses to meals and dietary macronutrient composition (Havel et al., 1999b; Havel, 2004; Perez-Matute et al., 2005). The effect of insulin to stimulate leptin gene expression and secretion (Mueller et al., 1998) and the transcriptional activity of the leptin promoter (Moreno-Aliaga et al., 2001) is mediated by insulin's actions to increase adipocyte glucose utilization. Furthermore, insulin shifts glucose metabolism from anaerobic metabolism to lactate to mitochondrial oxidation and this metabolic change appears to contribute to the effects of insulin to stimulate leptin production (Havel et al., 1999a; Mueller et al., 2000). Several studies have also suggested a role of insulin in the regulation of adiponectin secretion although data were conflicting (Halleux et al., 2001; Fasshauer et al., 2002).

Adiponectin has been shown to be a target gene of the transcriptional factor PPARγ (Iwaki et al., 2003), and several studies have demonstrated that the thiazolidinediones, selective ligands of PPARγ used in treatment of type 2 diabetes, increase adiponectin mRNA levels (Combs et al., 2002). Therefore, we also examined the effects of CLA on PPARγ gene expression in cultured rat adipocytes.

2. Materials and methods

2.1. Materials

Media (Dulbecco's modified Eagle's medium, DMEM), minimal essential medium amino acids, penicillin/streptomycin, fetal bovine serum (FBS), and nystatin were purchased from Gibco-Invitrogen Life Technologies (Carlsbad, CA, USA, Grand Island, NY, USA). Bovine serum albumin fraction V, 4-(2-hydroxyethyl)-1-piperazineethansulfonic acid (HEPES), insulin and CLA (40% of 10t12c and 40% of 9c11t) were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Collagen (Vitrogen 100) was obtained from Cohesion Technologies (Palo Alto, CA, USA). ^{14}C-glucose was obtained from Perkin-Elmer Life Science (Boston, MA, USA). Type I collagenase was purchased from Worthington Biochemical Corporation (Lakewood, NJ).

2.2. Animals

Male Wistar rats (250–280 g) obtained from Applied Pharmacobiology Center (CIFA-Spain) were used to provide adipocytes isolated from the epididymal fat depot. The animals were housed in cages in temperature-controlled rooms (22 ± 2 °C) with a 12-h light:12-h dark cycle. All experimental procedures were performed according to National and Institutional Guidelines for Animal Care and Use and approved by the Ethical Committee for Animal Care and Use at the University of Navarra, Spain. Animals were euthanized, and epididymal adipose tissue was removed.

2.3. Adipocyte isolation and culture

Adipocytes were isolated from epididymal fat depots. Epididymal fat was minced and digested in HEPES buffer (pH 7.4; containing 5 mM D-glucose, 2% BSA, 135 mM NaCl, 2.2 mM $CaCl_2{\cdot}2H_2O$, 1.25 mM $MgSO_4{\cdot}7H_2O$, 0.45 mM KH_2PO_4, 2.17 mM Na_2HPO_4, and 10 mM HEPES) with type I collagenase (1.25 mg/ml per 0.5 g tissue) at 37 °C with gentle shaking for 30 min. The resulting cell suspension was diluted in HEPES-phosphate buffer and the isolated adipocytes were then separated from the undigested tissue by filtration through a 400 μM nylon mesh and washed three times. Isolated adipocytes were then resuspended in DMEM supplemented with 1% FBS and incubated for 40 min at 37 °C. The isolated adipocytes (150 μl of 2:1 ratio of packed cells to medium) were then plated on 500 μl of a collagen matrix (Vitrogen 100, Cohesion Technologies, Palo Alto, CA) in 6-well culture plates. After 50 min of incubation at 37 °C, the culture media containing 0 or 1.6 nM insulin and the different concentrations (0, 1, 10, 50, 100 and 200 μM) of the assayed CLA were added and the cells were maintained in an incubator at 37 °C in 5% CO_2 for up to 96 h.

2.4. Assays

Leptin concentrations in the medium were determined after 96 h of culture using a radioimmunoassay for rat leptin (Linco Research, St. Charles, MO). Adiponectin concentrations were analysed using a highly sensitive mouse/rat adiponectin ELISA kit (B-Bridge International Inc, USA). Glucose and lactate concentrations in the media were measured by an Autoanalyzer (Cobas Roche Diagnostic, Basel, Switzerland) (Moreno-Aliaga et al., 2002).

2.5. Analysis of mRNA

Leptin and PPARγ mRNA levels were determined by Northern blotting. The leptin cDNA probe was a 388-bp fragment of mouse leptin cDNA, which was kindly provided by Dr. Charles Mobbs (Mount Sinai School of Medicine, New York). The PPARγ2 cDNA probe (GenBank accession no. Y12882) was a 593-bp fragment of rat cDNA, and the primers used were 5′-TCTGATTATGGGTGAAACTC-3′ (sense 43–62) and 5′-TTTCTACTCTTTTTGTGGATC-3′ (antisense 592–612). The 18S ribosomal probe was obtained from Ambion

(Ambion, Austin, TX). RNA was extracted according to the Gibco Life Technologies procedure using Trizol (Life Technologies Inc., Grand Island, NY). The UV absorbance and integrity gels were used to estimate RNA. Leptin, PPARγ2 and 18S cDNA probes were labeled by random priming (Rediprime kit, Amersham, Buckinghamshire, UK) in the presence of ^{32}P dCTP (3000 Ci/mmol, Amersham). Unincorporated nucleotides were removed using NucTrap probe purification columns (Stratagene, La Jolla, CA). For each tissue sample, 7 μg of total RNA were fractionated by electrophoresis on a denaturing 1% agarose gel containing 2.2 M formaldehyde and 1× MOPS running buffer. One microliter of a 50 μg/ml ethidium bromide (Gibco BRL, Gaithersburg, MD) stock solution was added in order to check RNA integrity and even loading. After electrophoresis, RNA was transferred to nylon membrane (Duralon-UV, Stratagene, La Jolla, CA) by overnight capillary transfer and UV cross-linked (Stratalinker 1800, Stratagene, La Jolla, CA). Blots were then hybridized for 1 h at 68 °C in presence of labeled cDNA probe (2×10^6 cpm/ml Express Hyb solution, Clontech, Palo Alto, CA and 1×10^6 cpm/ml with the 18S cDNA probe). After washing at high stringency, blots were exposed to X-ray films with an intensifying screen at −80 °C. The expression level of 18S ribosomal RNA was determined and used as an internal control to correct minor variation in total RNA amount.

2.6. Lipolysis

Lipolysis was assessed by measuring glycerol release into the media at 96 h. Glycerol was determined using an Autoanalyzer following the manufacturer instructions (Cobas Roche Diagnostic, Basel, Switzerland).

2.7. Statistical analysis

The amount of carbon released as lactate per amount of carbon taken up as glucose over 96 h was calculated as Δ[lactate]/Δ[glucose], where Δ is the difference, and expressed as a percentage. The amount of glucose incorporated to CO_2 was calculated as (dpm collected on Whatman 1 strip) (total glucose)/total dpm and expressed as a percentage of total glucose utilized. The amount of glucose incorporated into lipid was calculated as [(dpm extracted in 1 ml chloroform) (total glucose)/total dpm] × 10 ml chloroform. This value was normalized over the amount of total lipid recovered from the well and expressed as a percentage of total glucose utilized.

The experimental results from each adipocyte suspension prepared from a single animal were analyzed in relation to a control well from the same suspension. For this reason, the statistical analysis of the data was performed by a repeated measures ANOVA followed by a Dunnet's post-test (GraphPad Prism, GraphPad Software Inc, San Diego, CA, USA). Results were also analyzed and expressed as the percentage of the control. All data were presented as mean ± S.E. Differences were considered as statistically significant at $P < 0.05$. Different animals were used to provide adipocytes for the measurements of glucose incorporation into lipid and glucose oxidation than those used for measurement of leptin and adiponectin secretion, glucose utilization and lactate production.

3. Results

3.1. Effects of CLA on leptin expression and secretion

Incubation of adipocytes with CLA at concentrations from 1 to 200 μM inhibited both basal (by 35–65%) and insulin-stimulated leptin gene expression (by 65–85%) at 96 h (Fig. 1A and B). CLA (1–200 μM) inhibited basal leptin secretion at 96 h by −30% to −40% ($P < 0.05$–0.01) (Fig. 1C). As expected, insulin at 1.6 nM increased ($P < 0.001$) leptin secretion (by ∼40%). Co-treatment with CLA at the higher concentrations (50–200 μM) in the presence of insulin decreased insulin-stimulated leptin secretion by −20% to −40% ($P < 0.01$) (Fig. 1D).

3.2. Effects of CLA on adiponectin secretion

CLA (50–200 μM) inhibited basal adiponectin secretion (268.0 ± 93.3 ng/ml) by −20% to −40% ($P < 0.05$–0.01) (Fig. 2A). However, co-treatment of adipocytes with insulin prevented the inhibitory action of CLA on adiponectin production, with the exception of a non-significant trend towards inhibition at the highest CLA concentration of 200 μM ($P = 0.08$) (Fig. 2B).

3.3. Effects of CLA on glucose utilization

CLA at concentrations of 50–200 μM decreased basal glucose utilization over 96 h by −20% to −30% ($P < 0.05$–0.01).

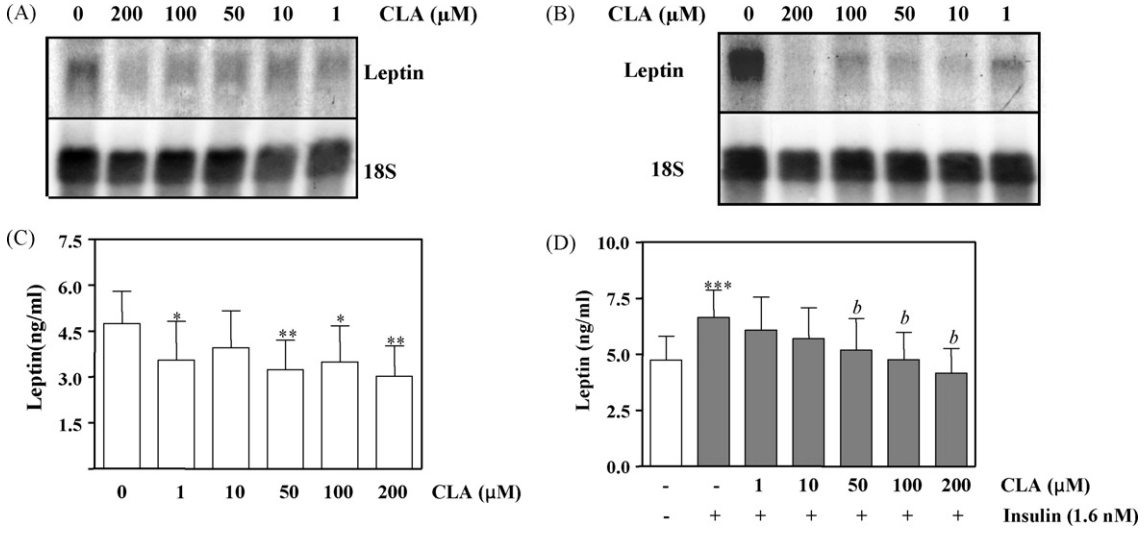

Fig. 1. Effects of CLA (1–200 μM) on basal (A) and insulin-stimulated (B) leptin mRNA expression, as assessed by Northern blots. The expression level of 18S ribosomal RNA was determined and used as an internal control to correct minor variation in total RNA amount. Results are representative of at least two independent experiments. Effects of different concentrations of CLA on basal (C) and insulin-stimulated (D) leptin secretion by isolated rat adipocytes over 96 h in culture ($n = 8$, mean ± S.E.) $^{*}P < 0.05$, $^{**}P < 0.01$ and $^{***}P < 0.001$ *vs.* control (cells without CLA and insulin treatment) and $^{b}P < 0.01$ *vs.* cells incubated with insulin alone.

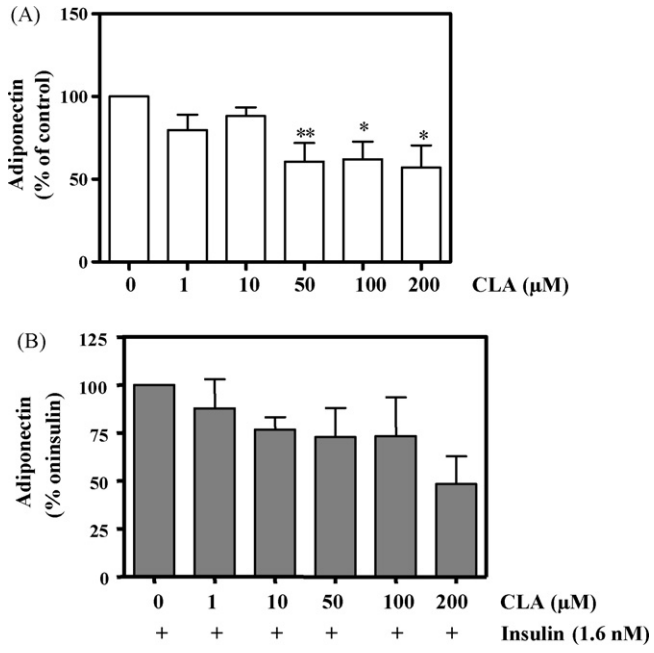

Fig. 2. Effects of CLA (1–200 μM) on adiponectin secretion by isolated rat adipocytes both in absence (A) and in presence of 1.6 nM insulin (B) ($n = 5$, media ± S.E., expressed as percentage of control). $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* control (cells without CLA and insulin treatment).

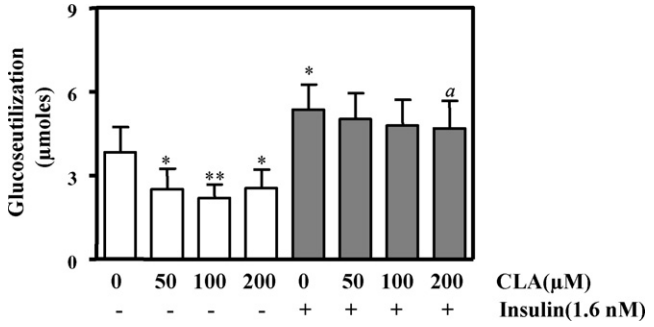

Fig. 3. Effects of CLA (50–200 μM) in absence or presence of 1.6 nM insulin on glucose uptake by isolated adipocytes over 96 h in culture. Data ($n = 6$, mean ± S.E.). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control (cells without CLA and insulin treatment) and $^{a}P < 0.05$ *vs.* cells incubated with insulin alone.

Insulin-stimulated glucose utilization was only significantly inhibited by CLA at the highest concentration examined, 200 μM (−16.4 ± 5.7%, $P < 0.05$) (Fig. 3). Adipocyte glucose uptake and insulin-stimulated but not basal leptin secretion were directly correlated (Fig. 4A and B). A strong positive relationship was found between glucose uptake and adiponectin secretion, both in the absence and in the presence of insulin (Fig. 4C and D).

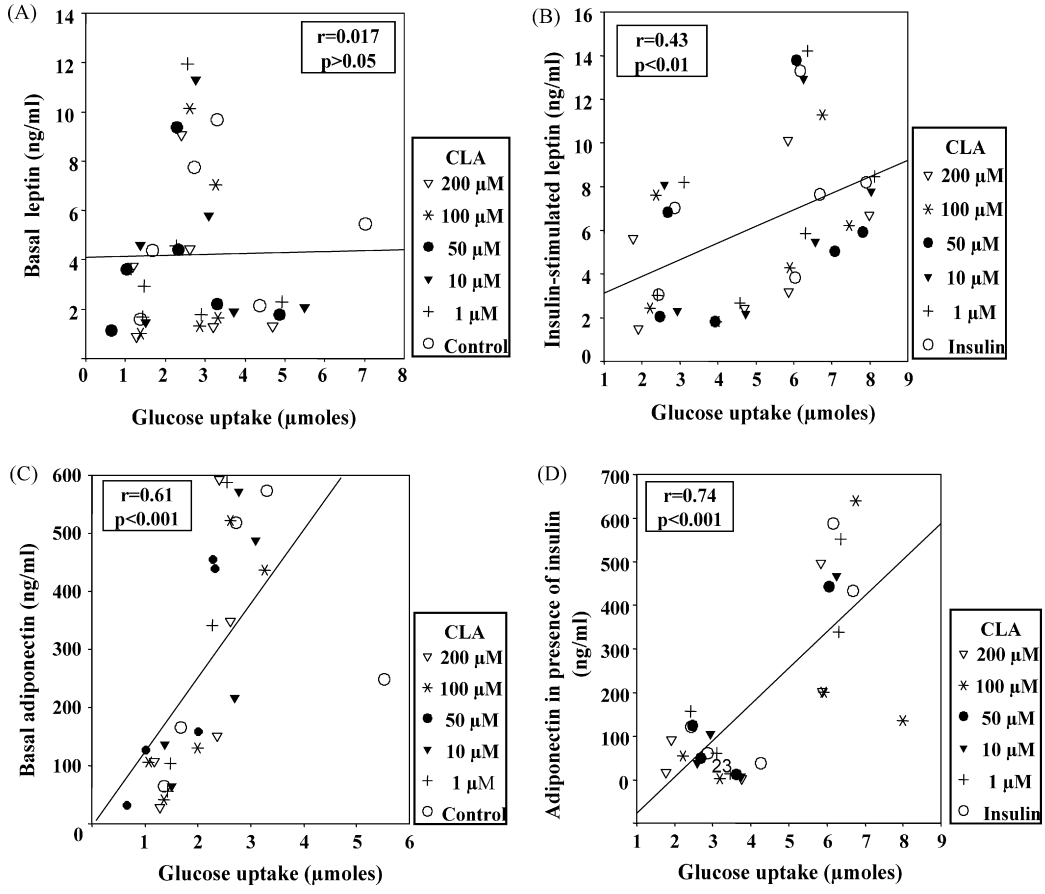

Fig. 4. Relationship between glucose uptake and both leptin (A and B) and adiponectin secretion (C and D) in the absence and in the presence of 1.6 nM insulin, respectively, in control and CLA-treated cells (1–200 μM) over 96 h of culture.

3.4. Effects of CLA on lactate production

CLA did not modify basal lactate production. Insulin decreased lactate production by $-27.0 \pm 6.6\%$ ($P < 0.01$). Co-treatment with CLA at the concentrations of 50 and 200 μM increased lactate production ($+25.2 \pm 7.7\%$, $P < 0.01$ for 50 μM and $+19.5 \pm 10.3\%$, $P < 0.05$ for 200 μM) (Fig. 5A).

The proportion of glucose carbon metabolized to and released as lactate over 96 h was significantly increased by CLA at the highest concentrations tested both in absence (50–200 μM) and presence of insulin (50 and 200 μM) (Fig. 5B). The percentage of glucose that was metabolized as lactate was inversely correlated with both leptin (Fig. 5C and D) and adiponectin production (Fig. 5E and F) in the absence and presence of insulin.

3.5. Effects of CLA on lipolysis

The effects of CLA on lipolysis were evaluated by determining the amount of glycerol released into the media over 96 h of culture. CLA (1–200 μM) decreased basal lipolysis by -15 to -40% ($P < 0.05$–0.01) (Fig. 6A). CLA also decreased the amount of glycerol released in the presence of insulin, but

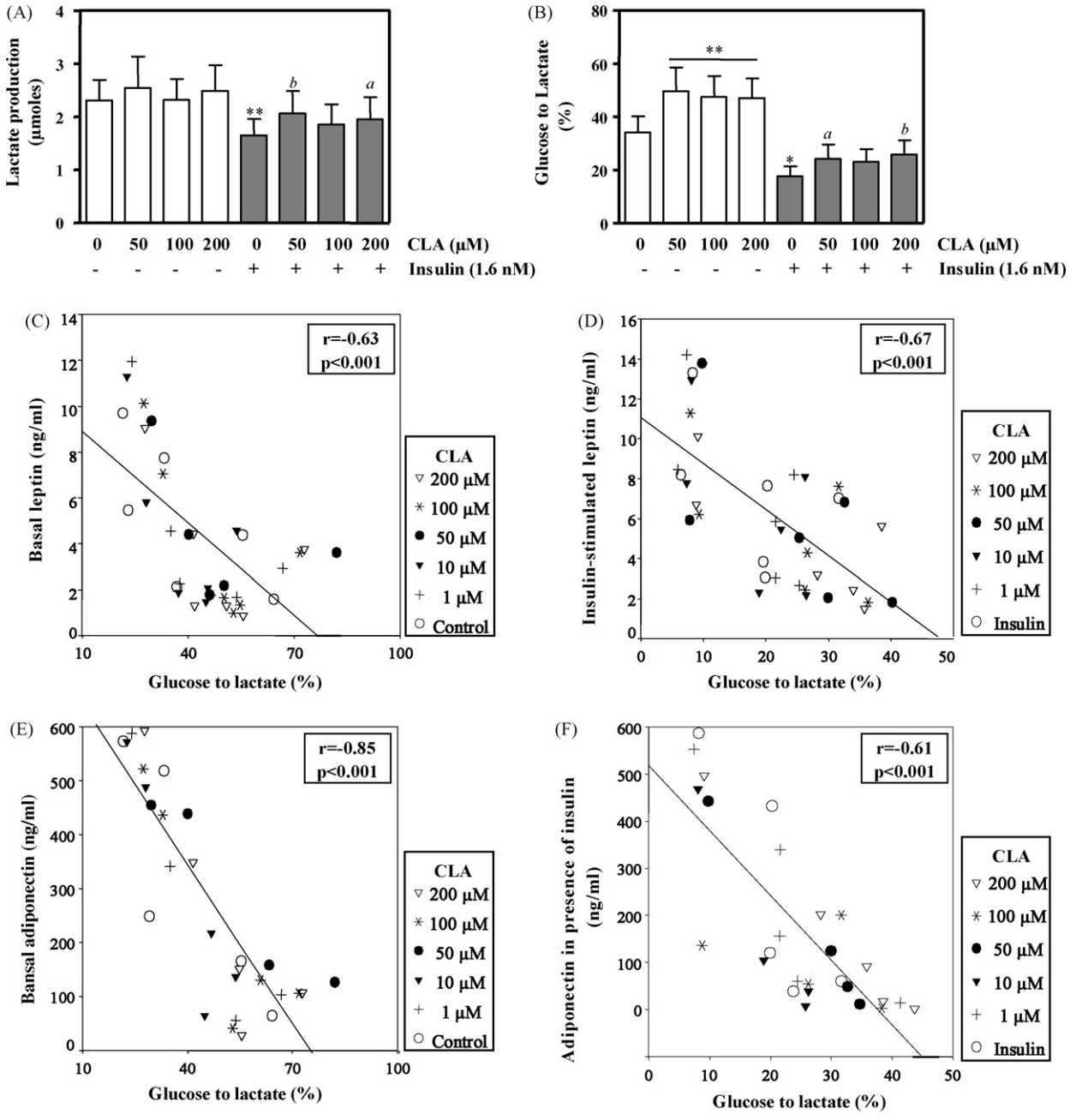

Fig. 5. Effects of CLA (50–200 μM) in absence or presence of 1.6 nM insulin on lactate production (A) and the percentage of glucose carbon released as lactate (B) by isolated adipocytes over 96 h in culture. Data ($n = 6$, mean ± S.E.). $^{*}P < 0.05$, $^{**}P < 0.01$ *vs.* control (cells without CLA and insulin treatment) and $^{a}P < 0.05$ and $^{b}P < 0.01$ *vs.* cells incubated with insulin alone. Relationship between the percentage of glucose metabolized to lactate and both leptin (C and D) and adiponectin secretion (E and F) in the absence and in the presence of 1.6 nM insulin, respectively, in control and CLA-treated cells (1–200 μM) over 96 h of culture.

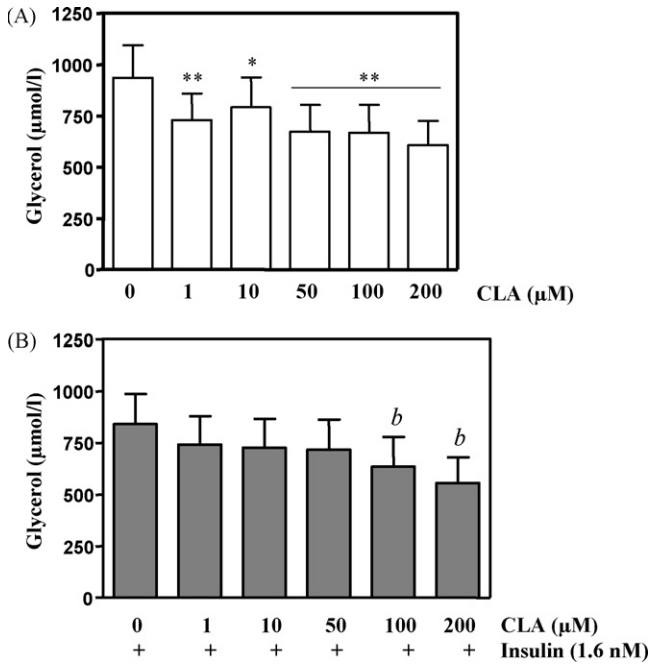

Fig. 6. Effects of CLA (1–200 μM) on lipolysis in absence (A) and presence of 1.6 nM insulin (B) by isolated rat adipocytes over 96 h in culture ($n = 8$, mean ± S.E.). $^{*}P < 0.05$ and $^{**}P < 0.01$ *vs.* control (cells without CLA and insulin treatment) and $^{b}P < 0.01$ *vs.* cells incubated with insulin alone.

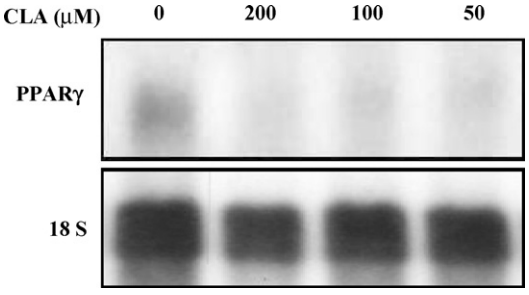

Fig. 7. Effects of CLA (50–200 μM) on basal PPARγ mRNA expression, as assessed by Northern blots. The expression level of 18S ribosomal RNA was determined and used as an internal control to correct for minor variations in total RNA amount. Results are representative of at least two independent experiments.

only at the highest concentrations tested (100 and 200 μM) (Fig. 6B).

3.6. Effects of CLA on PPARγ expression

CLA decreased PPARγ gene expression at concentrations of 50, 100 and 200 μM (~85% of inhibition) (Fig. 7).

4. Discussion

CLA consists of a group of positional and geometric isomers of linoleic acid. The *cis*-9, *trans*-11 (c9t11) isomer is the most abundant natural isomer present in ruminant tissue fats and in milk and other dairy products. Some studies have suggested that the *trans*-10, *cis*-12 CLA isomer (t10c12) is the isomer responsible for decreasing fat mass (Wang and Jones, 2004a,b), while the adverse effects on insulin sensitivity after CLA treatment are associated with the t10c12 isomer (Clement et al., 2002; Riserus et al., 2002). In the present study, we used a commercially available mixture of both CLA isomers, which consisted of ~40% of 9c11t isomer and ~40% of the 10t12c isomer. Hargrave et al. (2002) reported a decrease in body fat in mice treated with a mixture of CLA isomers as well as with the t10c12 CLA isomer alone.

The present results demonstrate a direct inhibitory effect of CLA on both basal and insulin-stimulated leptin gene expression and secretion. This suggests that CLA treatment can inhibit leptin production directly, and that decreases in circulating leptin levels reported in animal studies may not be solely a consequence of reduced fat mass (Yamasaki et al., 2003). This is supported by a human study in which CLA supplemented at 1% of energy requirements modestly decreased circulating leptin concentration, but did not affect body weight or body fat content (Medina et al., 2000). The inhibitory effect on basal leptin production seems to be specific for CLA since linoleic acid did not modify basal leptin secretion in primary cultured rat adipocytes (data not shown). Kang and Pariza (2001) also observed that 100 μM of the t10c12 isomer directly inhibited leptin secretion and gene expression in 3T3-L1 cells. However, Brown et al. (2004) reported a stimulatory effect of t10c12 CLA isomer on leptin gene expression in cultures of stromal vascular cells containing newly differentiated human adipocytes after 9 days of treatment. The disparity with our results is likely to be related to differences in the type of culture system employed (differentiating cultures of human SV cells *versus* collagen-fixed primary rat adipocytes).

Both a recent study in 3T3-L1 cells and a previous study in brown adipocytes observed that CLA isomers exhibited opposite effects on leptin. Thus, t10c12 CLA reduced while c9t11 CLA tended to increase leptin secretion or gene expression, respectively (Rodriguez et al., 2002; Ahn et al., 2006). The inhibitory action on leptin observed in our study using a mixture with similar concentrations of both CLA isomers, suggests that the presence of c9t11 CLA is not able to counteract the inhibitory action of t10c12 CLA.

Previous studies conducted in animals have described an apoptotic effect of CLA on white adipose tissue (Tsuboyama-Kasaoka et al., 2000). However, Brown et al. (2004) observed in primary cultured human adipocytes that cell numbers were not reduced after CLA treatment. Although we did not specifically measure apoptosis in these studies, the lack of effects of CLA on 18S ribosomal mRNA levels suggests that CLA did not exert an apoptotic action in the present studies. Therefore, the decrease in leptin secretion induced by CLA is more likely to be related to the inhibitory effect of CLA on leptin gene transcription rather than to a reduction of adipocyte number.

Several previous studies indicate that insulin-stimulated glucose metabolism rather than insulin *per se* is involved in the regulation of leptin production (Mueller et al., 1998; Moreno-Aliaga et al., 2001). Inhibitors of glucose uptake and metabolism inhibit insulin-stimulated leptin secretion in proportion to their metabolic effects (Mueller et al., 1998). One study reported that CLA supplementation decreased adipose tissue GLUT-4 (insulin dependent glucose transporter) gene expression in mice

meanwhile a significant increase of GLUT4 gene expression was observed in gastrocnemious muscle (Tsuboyama-Kasaoka et al., 2000). In this study, we observed that CLA decreased basal glucose uptake at every concentration studied. Insulin-stimulated glucose uptake was only inhibited by CLA at the highest concentration (200 μM), suggesting that insulin may override the inhibitory effects of CLA on glucose uptake. However, a strong relationship between glucose uptake and leptin production was observed in the presence, but not in the absence, of insulin suggesting that glucose uptake *per se* is not the main determinant of the inhibitory actions of CLA on leptin production. In fact, we have published data suggesting that aerobic metabolism of glucose beyond pyruvate, and not glucose utilization *per se*, is involved in the effects of glucose and insulin to increase leptin secretion (Havel, 2000; Mueller et al., 2000). In these experiments, leptin secretion was inversely related to the proportion of glucose anaerobically metabolized to lactate. In the present study, basal lactate production was unaffected by incubation with CLA, however, the percentage of glucose carbon metabolized to lactate was increased both in absence and in presence of insulin and was strongly inversely correlated to leptin production across all concentrations of CLA. These results suggest that the decrease observed in leptin secretion and expression induced by CLA may result from the effects of CLA to increase proportional anaerobic glucose metabolism. Previous studies have also suggested that glucose utilization stimulates leptin production by directing the metabolism of glucose to a fate other than anaerobic lactate production, mainly to oxidation or lipogenesis (Havel et al., 1999a; Mueller et al., 2000). In the present study, we did not observe any significant effect of CLA on both the percentage of glucose that is incorporated into triglyceride or that is oxidized to CO_2, although a moderate trend towards inhibition was observed in the proportion of glucose that was oxidized to CO_2 after CLA treatment (data not shown).

It has been suggested that increased lipolysis (and also elevated cAMP) is negatively related to leptin release by adipocytes (Fain and Bahouth, 2000; Moreno-Aliaga et al., 2002). Several studies have described a lipolytic effect of CLA in murine and human adipocytes (Park et al., 1997, 1999; Evans et al., 2002; Chung et al., 2005; Simon et al., 2005). However, other studies have not observed any effect of short-term intake of CLA on basal lipolysis in mouse adipose tissue (Xu et al., 2003). In the current study, basal lipolysis was significantly reduced during incubation with CLA at all concentrations tested and an inhibitory effect was also observed in the presence of insulin at the higher concentrations of CLA (100–200 μM). Thus, CLA-induced inhibition of leptin gene expression and secretion observed in the present study is unlikely to be related to increased lipolysis.

Adiponectin is an adipocyte hormone with anti-inflammatory and insulin-sensitizing properties. Circulating adiponectin concentrations are reduced in obese individuals and negatively correlated with increasing adiposity in humans and animals (Hotta et al., 2001; Stefan et al., 2002a; Cnop et al., 2003; Havel, 2004). The majority of studies conducted in animals demonstrate that CLA reduces body fat mass (DeLany et al., 1999; Wang and Jones, 2004a,b). However, it has been reported that CLA decreases adiponectin production in mice (Ohashi et al., 2004; Poirier et al., 2005), an effect opposite to what would be expected with a reduction in fat mass. This suggests that CLA may have a direct inhibitory action on adiponectin production. In the present study, we demonstrate for the first time that a mixture of CLA isomers inhibits adiponectin secretion from cultured isolated adipocytes. On the contrary, a recent study using separately both types of CLA isomers observed a slightly increase in adiponectin by the 9c11t isomer and no effects with the 10t12c isomer in 3T3-L1 cells (Ahn et al., 2006). The disparity observed between both studies could be related to a potential different action of CLA isomers on adiponectin when administered together than separately, but could also be related to type of culture system employed (3T3-L1 cells *versus* primary cultured adipocytes) and period of treatment.

The data suggest that a reduction in glucose utilization may be involved in the inhibitory actions of CLA on adiponectin. We observed a strong inverse relationship between glucose utilization by the adipocytes and adiponectin secretion, suggesting that glucose uptake may be necessary in order to secrete adiponectin. Furthermore, insulin was able to partially prevent the inhibitory effects of CLA on both glucose uptake and adiponectin secretion. In a recent study, adiponectin protein content in 3T3-L1 cells was decreased by glucose deprivation (Huypens et al., 2005). However, in this study incubation 3T3-L1 cells with metformin, which stimulates adipocyte glucose uptake (Mueller et al., 2000), also inhibited adiponectin protein expression and release, and the inhibition was related to activation of AMP-activated protein kinase (Huypens et al., 2005). In a previous study, we demonstrated that although metformin does increase glucose uptake, it also increases anaerobic metabolism of glucose to lactate and as previously discussed, this effect was inversely related to leptin secretion (Mueller et al., 2000). In the present study, and similarly to what was observed for leptin, the CLA-induced increase in the proportion of glucose metabolized to lactate was inversely correlated to adiponectin secretion. These results suggest that the glucose metabolism rather than glucose uptake *per se* is likely to be involved in the regulation of both leptin and adiponectin production by adipose tissue.

A number of studies have reported that the insulin-sensitizing class of drugs, thiazolidinediones, which are agonists of PPARγ, increase both adiponectin gene expression in adipose tissue and circulating adiponectin levels (Combs et al., 2002). It has been suggested that increased adiponectin production is a mechanism by which this class of compounds acts to improve whole-body insulin sensitivity (Yamauchi et al., 2001). Incubating cells *in vitro* with the t10c12 isomer of CLA decreased PPARγ gene expression in 3T3-L1 cells and in human preadipocytes (Granlund et al., 2003; Kang et al., 2003). Our data confirm that CLA also decreases PPARγ gene expression in primary cultured rat adipocytes and suggest that downregulation of PPARγ contributes to the observed inhibition of adiponectin secretion by CLA.

In summary, CLA inhibited leptin gene expression and secretion and adiponectin secretion *in vitro* in primary rat adipocytes. These inhibitory effects were correlated with alterations in glucose metabolism, specifically with an increase in the anaerobic

metabolism of glucose to lactate. The CLA-induced decrease in adiponectin secretion may also be mediated in part by inhibition of PPARγ gene expression. Inhibition of leptin and adiponectin production by CLA may contribute to the decreases in whole-body insulin sensitivity observed in CLA-treated animals and humans.

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